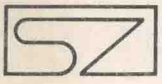
Olga Hudlická

Department of Physiology, University of Birmingham

muscle blood flow

its relation to muscle metabolism and function



SWETS & ZEITLINGER B.V. - AMSTERDAM
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Its relation to muscle metabolism and function

by

O. HUDLICKA

Department of Physiology,
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Photolitho-offset by Drukkerij N. Miedema & Co, Leeuwarden

ISBN 90 265 0131 5

PREFACE

This book is an attempt to summarize the present knowledge on circulation in skeletal muscle during activity and inactivity, together with my own experimental results in this field. I would like to thank all my collegues in different parts of the world who helped me in the course of preparation of the manuscript. My thanks are further due to Miss M. Cotter, B.Sc, and Mr. J.O.P. Chapman, B.Sc. who corrected the English, and to Miss R.O. Hore and Miss M. Hall for preparing the figures.

CONTENTS

Introduction 1

Chapter I. Anatomy and histology of muscle circulation 3
1. Anatomy of muscle blood vessels 3
2. Muscle capillary bed. Arterio-venous shunts 8
3. Capillarization in various muscles 9
4. The structure of the terminal vascular bed 16
a. Structure of arteries and arterioles 17
b. Structure of capillaries 20
c. Structure of veins 22
5. Innervation of muscle blood vessels 22
a. Vasomotor nerve fibres 22
b. Sensory nerve fibres 23
c. Vasodilator dorsal root fibres 25
d. Parasympathetric vasodilator fibres 25
e. Innervation of individual layers of the blood vessel wall 25
f. Capillary innervation 26
Summary 26

Chapter II. Basic mechanisms regulating muscle blood flow. Adaption of muscle blood flow to systemic circulatory changes 29
1. Resistance vessels 29
a. Basal tone of vessels. Physiology and metabolism of vascular smooth muscle 29
b. Vasomotor tone 34
c. Sensitivity to metabolites 35
d. Tissue pressure 35
e. Tone of striated muscle fibres 35
f. Perfusion pressure. Critical closing pressure 38
2. Capacitance vessels 39
3. Arterio-venous shunts. Dual circulation in skeletal muscle 41
4. Capillaries 44
a. Capillary blood flow 44
b. Capillary pressure and filtration 45
c. Diffusion through capillaries 47
d. Factors affecting capillary permeability 49
5. Circulation of lymph 50
6. Muscle blood flow during changes of systemic circulation 51
a. Changes in posture 51
b. Changes in oxygen saturation 51

c. Changes in circulating blood volume 52
d. Effect of stress and nociceptive stimulation 52
e. Effect of heat and cold 53
Summary

Chapter III. Humoral mechanisms affecting muscle circulation 55
1. Metabolites increasing muscle blood flow 55
a. Lactic acid and pH 55
b. Carbon dioxide 56
c. Lack of oxygen 56
d. Potassium 57
e. ATP and other metabolic products 57
f. Changes in osmolarity 58
g. Acetylcholine 59
h. Histamine 59
i. Kinins 60
2. Hormones influencing muscle blood flow. Catecholamines: adrenaline and noradrenaline 61
a. Adrenaline 61
b. Noradrenaline 63
c. Adrenal cortical hormones 64
d. Other hormones and active substances 64
3. Drugs affecting blood flow 64
a. Anaesthetics 64
b. Vasoconstricting drugs 65
c. Vasodilating drugs 65
d. The effect of vasoactive substances upon individual segments of the vascular bed 65
Summary 66

Chapter IV. Nervous regulation of muscle circulation 67
1. Sympathetic vasoconstrictor fibres 67
2. Sympathetic vasodilator fibres 71
3. Sensory nerve fibres 73
4. Organization of vasomotor centres 86
5. Reflex changes of vasomotor tone in muscles 88
6. Changes in muscle blood flow after sympathectomy 94
7. Changes in muscle blood flow after peripheral nerve section 96
Summary 106

Chapter V. Muscle metabolism and blood flow 107
1. Relationship between muscle metabolism and blood flow at rest 107
a. Metabolism and blood flow in different muscles 107
b. Blood flow as a factor regulating oxygen and substrate consumption 111
2. Blood flow and metabolism in working muscles 115

a. Changes in blood flow and oxygen consumption during tetanic and sustained contractions 116
b. Changes in blood flow and metabolism in rhythmically contracting muscles 117
c. Changes in transcapillary transport during muscular contractions 123
d. Causes of functional (postcontraction) hyperaemia 125
e. Blood flow in muscles in the course of training 129
3. Changes of blood flow and metabolism in inactivated muscles 130
a. Consumption and output of substrates and blood flow in denervated muscles 130
b. Consumption and output of substrates and blood flow in tenotomized muscles 135
Summary 139

Chapter VI. Disturbances of muscle circulation 141
1. Metabolic and functional changes in skeletal muscle due to reduced blood supply 141
a. Changes in metabolism 141
b. Functional changes 142
c. Morphological changes 142
d. Circulation in the occluded region 142
2. Recovery of circulation after released occlusion 143
Summary 148

Conclusions 148

References 153

Author Index 201

Subject Index 211

INTRODUCTION

It was not until many years after Harvey's discovery of the basic principles of blood circulation that skeletal muscle blood flow began to attract the interest of physiologists. It was apparently Hales (1733) who was the first to observe the movement of erythrocytes in the capillaries of the rectus abdominis of the frog and thus helped to open up new aspects of blood circulation – namely the passage of blood from arteries into veins. Special interest in muscle circulation was not displayed until it was discovered that only a few organs in the body exhibit such large shifts in blood flow as the skeletal muscle. During activity, muscle blood flow increases more than tenfold above the resting value, which is relatively small, and differs considerably in different muscles in relation to their function.

The increase of blood content in working muscle was apparently first described by Fontana (1785) and precise measurements were performed by Gaskell in 1877. The mechanisms causing this considerable increase in blood flow have been studied by a number of physiologists for almost a hundred years. Notwithstanding, this problem is still not clear and relations between the intensity of metabolism and blood flow represent one of the principal, hitherto not too clearly understood chapters of the regulation of muscle flow. Until very recently, people studying circulation in skeletal muscle were taking for granted that any muscle could be taken as representative for the whole bulk of muscle tissue. Muscle physiologists and biochemists knew, however, for a long time that the differences between skeletal muscles in one and the same animal may be almost as great as differences between skeletal and cardiac muscle. There are static muscles concerned in the maintenance of posture which contract very slowly, have high aerobic and relatively low anaerobic metabolism and high content of myoglobin which is partly responsible for their red colour. Dynamic muscles involved in rapid phasic movements contract very fast, have very low aerobic and high anaerobic metabolism and low content of myoglobin (and are known as white muscles). In between these two extreme types are a number of transitions, since most muscles are composed of muscle fibres of both types, and since even individual muscle fibres can be intermediate. It is hoped that some of the discrepancies described in the regulation of blood flow so far by different authors may be explained if the fact of the great variability of muscle tissue is taken into account.

Changes in blood flow in active muscles have been studied very extensively. Much less attention was paid to changes in blood flow in inactive (immobilized or atrophied) muscles. The experimental material presented in this book shows that, contrary to what is generally believed, inactivity, or even atrophy, does not mean diminished blood supply.

Apart from activity and inactivity the tone of skeletal muscle fibres themselves is involved in regulation of the blood flow, and mechanical conditions inside the muscle can thus influence flow – a situation unique to skeletal muscle.

Nervous factors play a very important role in the regulation of muscle blood flow. Muscle blood vessels are innervated, as are vessels in other parts of the body, by sympathetic, adrenergic vasoconstrictor fibres. Besides this, however, they also receive a sympathetic, cholinergic vasodilator innervation, the significance of which has recently, at least partly, been elucidated. Furthermore, muscle circulation may be considerably modified by changes in the activity of somatic motor nerve fibres and blood flow may also be regulated by higher nerve centres by a reflex route, if the changes occurring in the muscle are being signalled by sensory nerve fibres. The role of the sensory outflow and altered activity of motor nerve fibres in the regulation of muscle blood flow has hitherto been studied very inadequately and in this monograph an attempt is being made to answer, at least in part, some of the questions involved.

Muscle blood flow is substantially affected, as in other organs, by the basal blood vessel tone and the autoregulatory activity of the blood vessel wall, and by humoral factors which include both the effect of hormones and of a number of metabolites.

The original purpose of this book was to summarize the present knowledge of circulation in skeletal muscle in special relation to its metabolism and function. However, due to unforeseen circumstances, it is going to be published much later than originally intended, and it is very difficult to include all the new findings (presented in almost a hundred papers in 1972 alone). If the monograph helps to give a revision of the subject to people interested in muscle blood flow, and does not increase too much the already existing confusion, I would be satisfied.

I

Anatomy and histology of muscle circulation

1. Anatomy of muscle blood vessels

A schematic picture of blood vessel distribution in skeletal muscle was first put forward by Spalteholz (1888): a muscle is usually supplied by one or several arteries which are perpendicular or diagonal to the muscle fibres. They form a network visible macroscopically (1st order), then a microscopical network (2nd order) which then branches into capillaries which run parallel to muscle fibres. Venules and larger veins usually accompany the arteries. Both in the arterial and venous part of the vascular bed, there are numerous connections between various vessels (Rous et al., 1930): thus arterio-arterial and veno-venous anastomoses form a network. The "macromesh" of this network in the latissimus dorsi, trapezius and serratus ant. of man (corresponding to the 1st order network of Spalteholz) is about 1-2 cm wide, 1-5 cm long and arterioles form arcade-like branches which give rise to a finer network – the "micromesh" (Saunders et al., 1957) which corresponds to the 2nd order network. Spalteholz's pictures of the vascular bed obtained by histological techniques were recently confirmed by intravital microscopic observations in rat cremaster muscle by Grant (1964, 1968) and Baez (1969), in rat spinotrapezius muscle by Zweifach and Metz (1955) and Stingl (1971) and in cat tenuissimus by Eriksson and Myrhage (1972) (Fig. 1). The diameter of the supplying arteries varies greatly in different muscles and species (the supplying artery in cat tenuissimus in only 110μ wide).

The number of supplying arteries is different in various muscles as well, and is decisive for the formation of collateral circulation. From this point of view, muscles may be classified into three groups (Campbell and Pennefather, 1919):

a. Muscles supplied from several sources with numerous anastomoses between individual regions. This group includes the deltoid, pectoralis major, scapularis, biceps brachii, triceps brachii, anterior branchialis, adductor magnus, gluteus medius and minimus and to a certain extent also the soleus, vastus internus and externus and deep calf muscles. The ligation of one afferent vessel leaves the blood supply practically unaffected, since collateral circulation is sufficient to maintain it. According to Blomfield (1944) and Clarke et al., (1945), tibialis anterior also belongs here.
b. Muscles supplied by one to three arteries with a relatively small number of anastomoses between individual regions: e.g. gluteus maximus, rectus femoris, semimembranosus, semitendinosus, biceps femoris and sartorius. Interruption of the blood supply of either of the two main branches results in ischaemia of a large portion of the muscle (Clarke et al., 1945).
c. Muscles with a single main artery: crureus, gracilis and both the medial and lateral head of the gastrocnemius. Occlusion of this main artery leads to complete muscle ischaemia.

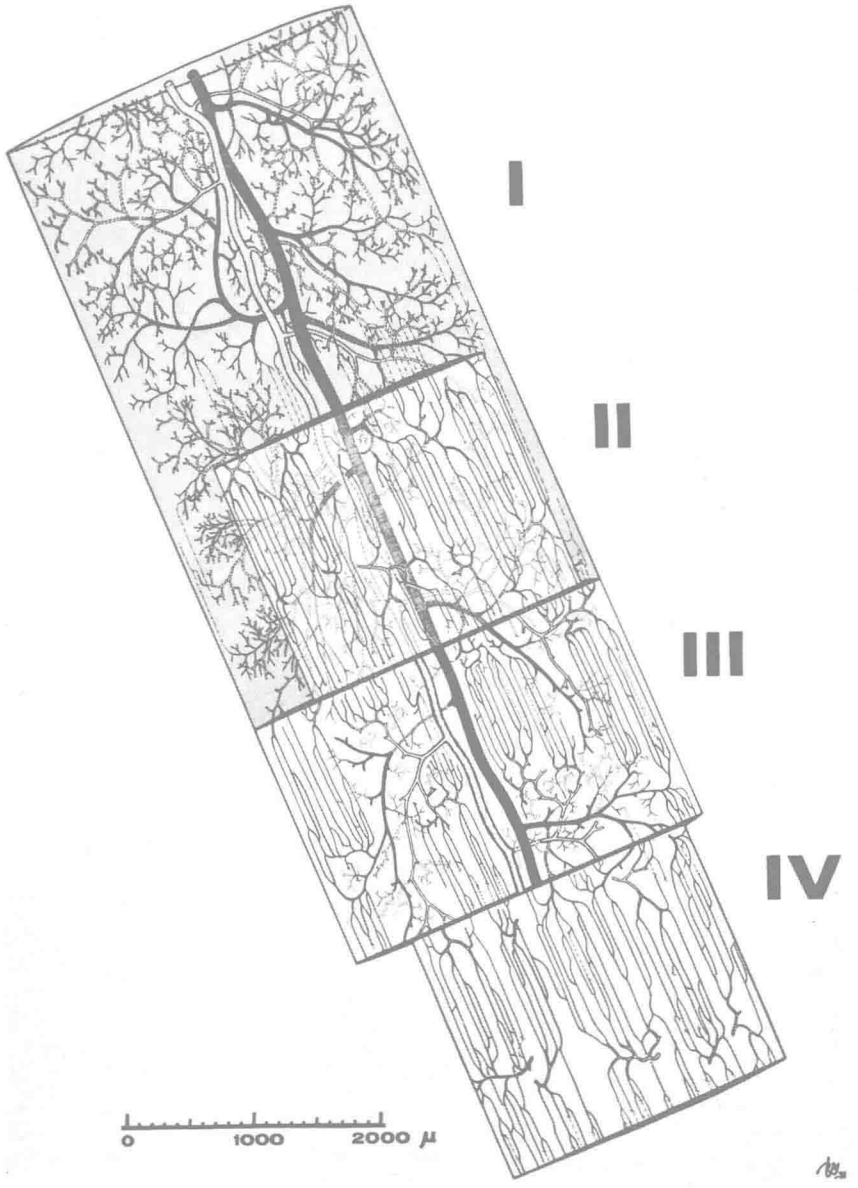

Fig. 1. A detailed schematic representation of the vascular architecture of the cat tenuissimus muscle. Arterial vessels open, venous vessels full. Sections (each about 100μ deep) are made into the muscle at II, III and IV.
From Erikson & Myrhage (1972) by the courtesy of the authors and Acta physiol. scand.

Individual variations are apparently considerable (Blomfield, 1944), in fact, so great that Clarke considers the gracilis to belong to the group of muscles with very good collateral circulation.

The number of arteries supplying individual muscles does not seem to affect blood flow and consequently the oxygen supply to muscle cells. It could be assumed that muscles with a greater intensity of oxidative metabolism (the so-called red, static muscles) receive a more extensive blood supply than white, dynamic muscles. Boerhaave (1775) first observed that the blood content of red muscles was higher than that of the white. However, muscles receiving three arteries (group a), for example, may belong either to the dynamic or the static muscle group (Denny-Brown, 1939) and this also applies to muscles in the other categories (the gastrocnemius of the dog being more a static muscle, the gracilis predominantly a dynamic muscle). In fact Stingl (1970) was unable to find any difference in the number of arteries supplying a muscle in relation to its type or function. The number of large arteries is evidently not decisive for the extent of muscle blood supply in relation to its function and metabolism. On the other hand, however, considerable differences are found between red (static) and white (dynamic) muscles in the arrangement of vessels on the level of microscopic network.

The structure of the vascular bed in different muscles was first studied by Ranvier (1874) and later by Meyer (1875) and recently reinvestigated by Hammersen (1964) (Fig. 2). After injecting Berlin blue, these authors found that capillaries in white muscle (adductor magnus of the rabbit) form elongated loops parallel to the muscle fibres, the transverse branches encircle individual fibres.

In red muscles (semitendinosus), the loops are of equal length and breadth, but longitudinal branches of the network form variable sinusoids and transverse branches have varicose dilations 17-25μ diameter, (i.e. they are 2-3 times wider than the capillaries themselves) and the volume of the vascular bed is naturally greater. Similar dilatations occur at the sites where several capillaries join to form a venule (Ranvier, 1874). Furthermore, Meyer (1875) observed that while in white muscles the blood vessels begin to orientate themselves along muscle fibres very soon after entering the muscle, in red muscles the vessels enter the muscle at right angles to the muscle fibres and tend to form anastomoses, loops and a richer capillary network. More recent reports have confirmed and extended these results. It has been shown by Lee Ching-Yen (1958) that sack-like capillary dilatations appear in places where capillaries join to form the venule in red muscles only and develop (in rabbit) within the first month after birth. Visualization of capillaries by histochemical methods has enabled workers to demonstrate the sinusoidal course of individual capillaries particularly around the muscle fibres with high activity of oxidative metabolism (Romanul, 1965) (Fig. 3) in close connection with the subsarcolemmal concentration of oxidative enzymes. Muscle fibres with predominantly glycolytic type of metabolism are supplied by fewer and different capillaries. These findings offer a new explanation to the question why there is such a variation in the number of open capillaries in skeletal muscle, and whether or not A-V shunts described for instance in mesenterium by Zweifach (1936) do exist in skeletal muscle.

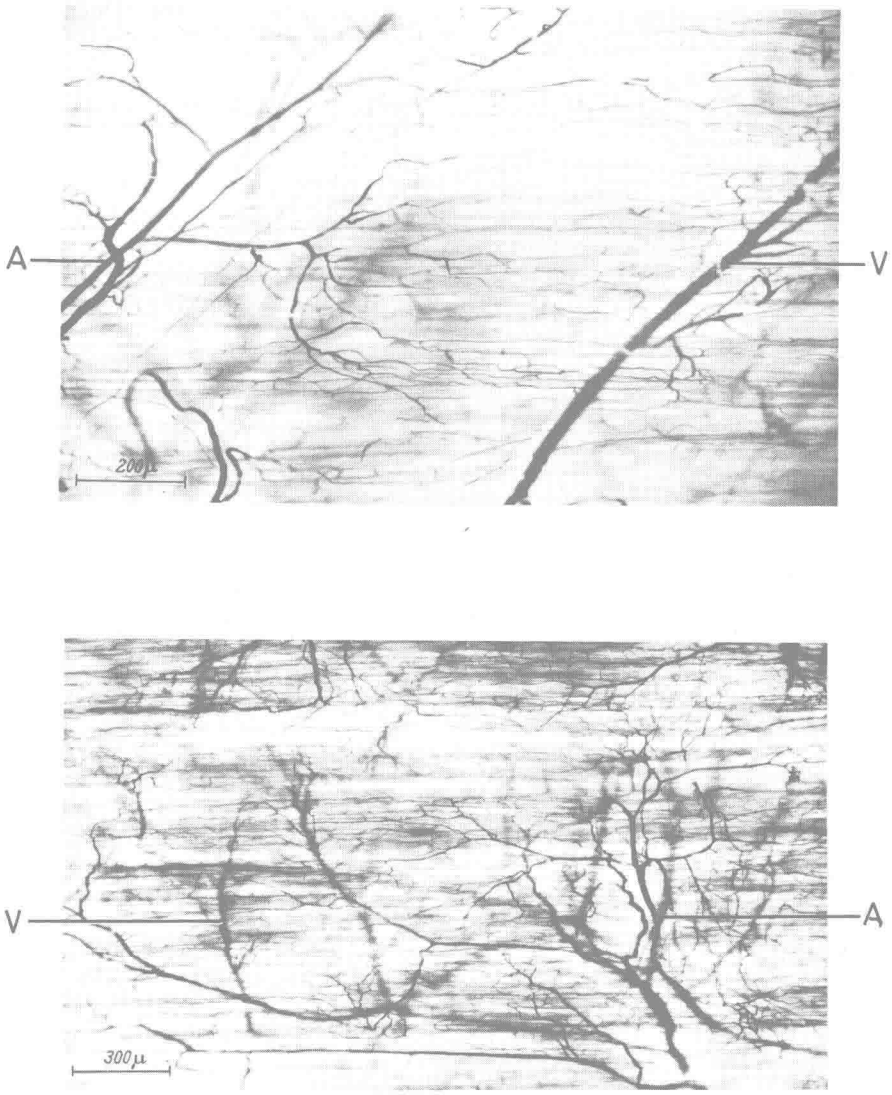

Fig. 2. Vascular supply of the semitendinosus ("red") and adductor magnus ("white") muscles of the rabbit. Semitendinosus above, x 45, adductor magnus below,x 65. Blood vessels injected with Berlin blue with gelatine.
From Hammersen (1964) by the courtesy of the author.

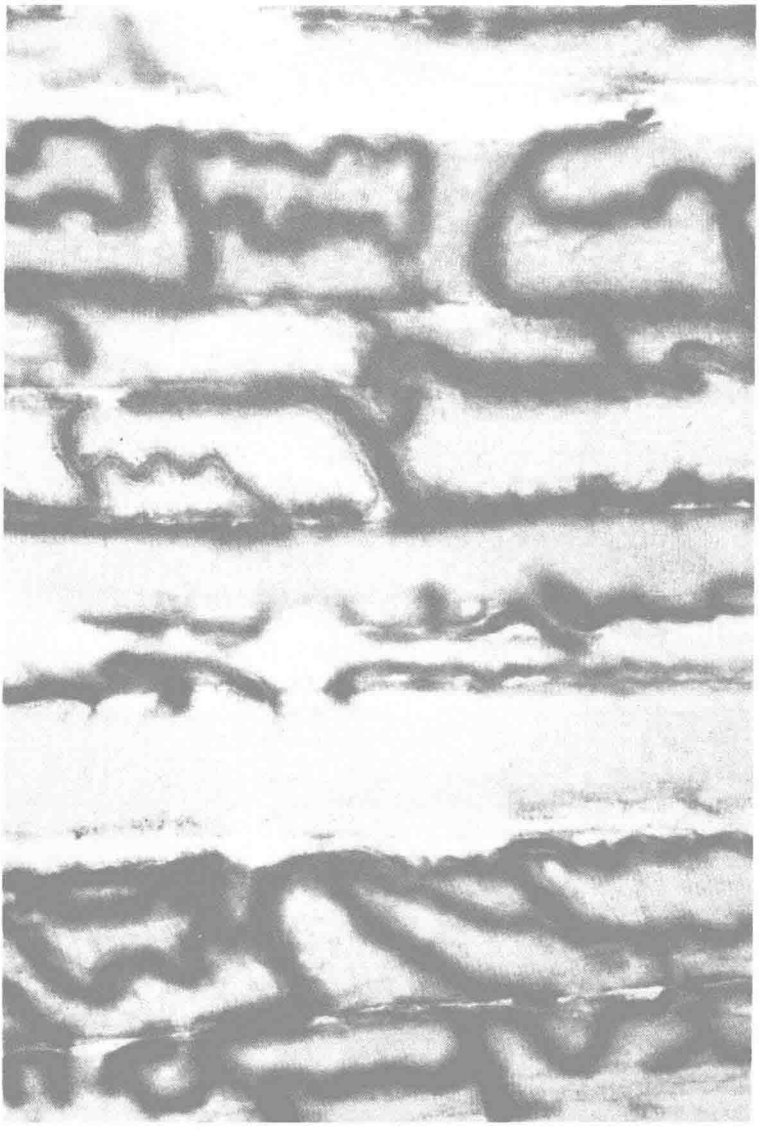

Fig. 3. Longitudinal section of rat soleus incubated for alkaline phosphatase. x 190. From Romanul (1965), by permission.

2. Muscle capillary bed. Arterio-venous shunts

The muscle capillary bed differs from that of most other organs by possessing a relatively small number of open capillaries at rest. However, during muscle contractions the number of open capillaries increases several times – eight times according to Krogh (1922) – two to three times according to more recent reports (Martin et al., 1932; Wright and Sonnenschein 1965; Renkin et al., 1966 and others). This large difference in the number of open capillaries may be explained according to Krogh's concept by assuming that most capillaries are closed at rest because the smooth musculature of the precapillary sphincters is contracted preventing the entry of blood into them. During work the tone of these smooth muscle cells relaxes due to metabolite accumulation and the vascular bed opens up. On the other hand Zweifach (1936, 1939), studying primarily the frog and mouse mesenterium and skin, showed that at rest blood flows mainly through arterio-venous bridges (shunts) or thoroughfare channels, the diameter of which differs only slightly from that of the capillaries, while true capillaries open up during functional activity. A-V shunts were described in the trapezoid muscle of the rat by Zweifach and Metz (1955), in frog muscles by Nikiforova and Shoshenko (1963) and the rat cremaster muscle by Grant (1964), but the evidence against their existence in skeletal muscle seems to be more convincing (Boyd, 1952; Staubesand, 1955; Wiedemann, 1963; Hammersen, 1970 and Stingl, 1970). Algire and Merwin (1955) found them only exceptionally in the back muscles of the rat, and Eriksson and Myrhage (1972) in the tenuissimus muscle of the cat. Grant and Wright (1970) described A-V shunts in muscle tendons. The discrepancies might be partly explained if we take into consideration the fact that muscles are composed of different proportions of red and white muscle fibres. Since red muscles seem to be supplied by capillaries which are constantly open, while white muscles are supplied by capillaries with intermittent blood flow (Gray, 1971), it could be that wherever thoroughfare channels were observed they were mistaken for capillaries supplying red muscle fibres. True A-V anastomoses (A-V-A) with contractile walls, the existence of which is still doubtful, do perhaps exist in the connective tissue between the muscle fibres (as they do in the skin). A certain (though small) number of A-V-A was described in the septa between individual muscle fibre bundles by Hammersen (1964) and in muscle fascia by Eriksson and Myrhage (1972). This observation is in accord with some physiological measurements which had led to the conclusion that if A-V-A do exist in the muscle, they are probably in the septa: as blood pressure drops to 40 mmHg, blood still flows through this region, while the muscle parenchyma becomes ischaemic (Illig, 1961). Walder (1953, 1955) and Barlow et al. (1958, 1959) pointed out that there may be a different circulation in the connective and muscle tissue; these authors did not consider this to be due to A-V-A, since they were unable to prove their existence by intravital staining. Because the clearance of ^{24}Na was much faster after exercise as compared with the plethysmographic values, they supposed that there are two vascular beds in skeletal muscle. It was then possible (with the aid of autoradiography) to localize the region of the slow circulatory component to the intramuscular septa and tendons, while the faster component represents circulation between the muscle fibres. Slow and fast components of blood flow in skeletal muscles were observed by other workers (see e.g. Freis et al., 1957; Shepherd and Warren, 1960; Kjellmer et al., 1967) but not in

connection with a definite anatomical localisation. It could be explained by different capillarization of different muscles, or even of different areas within one muscle.

3. Capillarization in various muscles

Krogh (1919a) was the first to show differences in the number of capillaries in various muscles, by injecting India ink. In 1 mm^2 of muscle tissue, he found 1350 capillaries in the horse gastrocnemius, 2630 in the dog semimembraneus, about 400 capillaries in the cod and frog muscles, 90 in the rectus abdominis, 2500 capillaries in the diaphragm and 2840 in the masseter of the guinea-pig. Subsequent reports (Table 1) confirmed these findings and corrected some inaccuracies due to artifacts which result from different degrees of retraction in verious preparations when capillaries are being calculated per unit area (for example, Stoel (1925) pointed out that the white muscle of the rabbit retracts more on fixation than the red muscle).

Another source of discrepancy between different findings is the fact that so called red muscles have much smaller diameters of muscle fibres than white muscles, and the diameter of muscle fibres also differs from one animal to another. For this reason the number of capillaries in subsequent works was calculated in relation to the number of muscle fibres, as determined in muscle cross-section. It may be concluded that white muscles contain less capillaries per muscle fibre than the red (less than 1.0 in the white and about 2 or more in the red muscle) and that this ratio develops gradually during ontogenesis. For example, in a 5-day-old starling embryo there is only one capillary per 15-20 fibres in the red pectoralis thoracicus muscle, in an 8-day-old embryo one capillary per 15 fibres, in a 19-day-old embryo 1.5 capillaries per fibre and in the adult 4-6 capillaries per fibre. In chickens the development is slower – the ultimate number of 0.5 capillaries per fibre in white muscles is not reached until 100 days of age (Bösiger, 1950). The development of capillarization of the cardiac muscle has an analogous course (Rakušan et al., 1965). While in the 5-day-old rat there is one capillary per four muscle fibres, this ration is 1:1 in the adult. In old people the number of capillaries per muscle fibre is smaller mainly because the diameter of muscle fibres decreases and there are therefore more muscle fibres for the same number of capillaries in a given area (Paříkzková et al. 1971).

A very detailed study of the capillarization of various muscles has recently been made by Romanul (1965) using the alkaline phosphatase technique which is localized in capillary endothelium. This author counted all capillaries (not only the open capillaries) in the soleus and gastrocnemius of the rat, plantaris of the rabbit and in human bioptic material and found that fibres with high oxidative metabolism (with high cytochrome oxidase and succinicdehydrogenase activity) are surrounded by a greater number of capillaries (six to eight) than fibres with low activity of oxidative enzymes (where there is one, or less than one, capillary per muscle fibre). Hence the distribution of capillaries in mixed muscles (e.g. gastrocnemius, plantaris) is very irregular, since the "red" fibres are surrounded by a greater number of capillaries than the "white" fibres. Romanul's findings were confirmed by Hammersen (1968), Mai et al., (1970) and others. In this way it is possible to explain some older observations (Lindgren, 1934; Martin et al., 1932) concerning the uneven filling of

Table 1. Capillary density in different muscles

Author	Animal species	Muscle	Number of capillaries per mm^2	Number of capillaries per muscle fibre	Diameter
Krogh (1919a)	guinea-pig	rectus abdominis	85		3.5 μ
		diaphragm	2500		5.0 μ
		masseter	2840 ± 100		
	dog	semimembranosus	2630 ± 51		
	horse	gastrocnemius	1350 − 31		
	frog, cod	sartorius, submaxillaris	300 ± 500		
Bösinger (1950)	chicken	white pectoral muscles		0.5	
	quail	white pectoral muscles		1-2	
		red pectoral muscles		3-5	
	starling	red pectoral muscles		4-6	
Duyff & Bauman (1927)	rabbit	white: gastrocnemius med	1352	1.80	
		gastrocnemius lat	1335	1.74	
		adductor magnus		1.52	
		biceps femoris	1065	1.40	
		red: soleus	1695	2.26	
		semitendinosus	1198	2.33	
		cruralis	1182	1.74	
Smith & Giovachini (1956)	rabbit	white: gracilis			
		semimembranosus			
		tibialis ant.			
		extensor dig. long.		0.5 (0.2-0.9)	
		red: soleus			
		adductor longus		1.7	
Stoel (1925)	rabbit	white: adductor magnus	1550	0.7	2.5 μ
		red: semitendinosus	790	1.2	5.2 μ